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Do Enallagma exsulans from Streams and Lakes Show Patterns of Divergence?

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Do *Enallagma exsulans* from Streams and Lakes Show Patterns of Divergence?

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by

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ABSTRACT

Divergent selection across heterogenous environments could lead to adaptive divergence in populations resulting in potential local adaption. These populations have phenotypic differences that are fitness related and make native individuals more fit than non-native individuals. My research focuses on a species of damselfly, *Enallagma exsulans*, to explore local adaptation and morphological differences as a result of divergent selection or plasticity. My first study explored potential local adaptation of wild caught stream and lake *E. exsulans* using a reciprocal transplant design, a classic approach for this objective. The stream and lake sites chosen were on a small spatial scale allowing for potential gene flow among populations, a process that could hinder local adaptation. In the second part of my research, I reared stream and lake *E. exsulans* in a common garden and transplanted them into stream and lake environments. I expected to find that native individuals had higher fitness, measured as growth rates, than non-native individuals indicating local adaptation. Unfortunately, I was unable to collect any results due to a storm damaging my experimental set-up. There are still important questions about local adaptation occurring at small spatial scales with potential for gene flow, and if plasticity is another mechanism for coping with changing environments. In the next part of my study, I used individuals raised in a common garden environment for a small scale mesocosm reciprocal transplant replicating the field study. All larvae lost body mass, no matter the origin of the individual or the condition under which it was tested. I also completed geometric morphometric analyses of wild caught individuals from both stream and lake environments and common garden reared individuals to determine if morphological differences are the result of divergent selection between populations. In wild-caught individuals, I found significant differences in body and lamellae shape between lake and stream populations suggesting divergent selection. In common garden individuals, I did not detect significant differences, suggesting morphological divergence is not genetically based. Last, I completed behavioral

assays with common garden individuals placing larvae into stream and lake conditions and scoring behavior, but no results were significant between lake and stream populations.

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INTRODUCTION

In heterogenous environments, some populations diversify while others do not. This has important implications for how biological diversity is maintained and increases. A population's ability to diversify depends on the balance between selection and gene flow in the different environments and the extent of the species' phenotypic plasticity (Hendry, Taylor, & McPhail, 2002). Selection has to be strong enough to overcome gene flow, but gene flow brings more genetic variation into a population for selection to act. The result of this balance between two evolutionary forces is local adaptation (Blanquart, Kaltz, Nuismer, & Gandon, 2013; Hereford, 2009; Moore & Hendry, 2005; Slatkin, 1987). When a population is locally adapted, the native individuals are more fit in that environment than non-native individuals entering the same environment. Reciprocal transplants are a classic approach for detecting local adaptation (Kawecki & Ebert, 2004) and I attempted to set-up such an experiment between lake and stream populations of *Enallagma exsulans*. To document local adaptation, I intended to quantify differences in growth rates, a measure of fitness in damselflies. Common garden experiments, where individuals representing populations adapted to different environments are raise under identical conditions, are another approach to assess local adaptation, but they eliminate environmental effects and hence allow to focus on phenotypic plasticity (Sharpe, Räsänen, Berner, & Hendry, 2008).

Morphological divergence can be the result of environmental heterogeneity and divergent selection (Hendry et al., 2002; McPeek, 2006; Taugbøl, Junge, Quinn, Herland, & Vøllestad, 2014). A way to investigate morphological differences between populations is shape analysis using a geometric morphometric approach (Cooke & Terhune, 2015). Geometric morphometrics employs Cartesian coordinates to encapsulate shape variation instead of the linear measurements used in traditional morphometrics (Adams, Rohlf, & Slice, 2004; McKeown & Schmidt, 2013). Changes in morphology or behavior can be the result of an adaptive response to environmental change, or represent phenotypic plasticity. For example, in a stream environment, certain features may be more advantageous such as wider and longer lamellae for better swimming strength and ability. Larvae with those types of lamellae may be better swimmers in flowing water, and allowing them to change their foraging behavior increasing their efficiency to catch prey. These changes can be the result of local adaptation in each environment or phenotypic plasticity, where organisms respond to the present environment by changing their expressed phenotype.

In Chapter 1, I aimed to determine if stream and lake *E. exsulans* are locally adapted to their environment using a reciprocal transplant design and a common garden experiment. In Chapter 2, I aimed to determine if there were morphological differences between stream and lake individuals from across the region as a result of divergent selection. I also used common garden reared larvae for this part of the study to determine if there is morphological divergence independent of environmental effects. I completed a small-scale reciprocal transplant with individuals raised in a common garden environment to determine if damselflies from lake or stream populations show fitness differences when exposed to lake and stream and conditions. Last, I analyzed the behavior of the common garden raised individuals when placed in a flowing stream or a still lake environment. All of these experiments were aimed towards addressing the overall question of determining if there is local adaptation or population divergence between stream and lake *E. exsulans*.

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CHAPTER 1: LOCAL ADAPTATION OF STREAM AND LAKE *ENALLAGMA EXSULANS*

ABSTRACT

Divergent selection can lead to population divergence when different environments select for different phenotypes. When these phenotypes have a heritable basis, selection can result in local adaptation. However, high levels of gene flow, as might happen when populations occur in close proximity to one another, can prevent local adaptation unless selection is particularly strong. Stream bluets, *Enallagma exsulans*, provide an ideal organism to evaluate these ideas because individuals of this species occur in lake and stream environments that are in immediate proximity to one another with no geographic barriers. To examine if local adaptation occurs between populations found in paired streams and lakes, a reciprocal transplant experiment comparing per capita growth rates, as a measure of damselfly fitness, was used. To control for the effects of the environment and potential phenotypic plasticity, stream and lake damselflies were reared in a common garden and transplanted into their native and contrasting environments to determine if fitness stayed the same. Unfortunately, results from these experiments were not obtainable over the course of field work, because of severe storms that destroyed the experiments. Whether or not divergence with gene flow can occur over the kind of short spatial scales evaluated here remains an important question.

INTRODUCTION

Population level divergence suggests organisms are responding to environmental variation potentially leading to local adaptation (Chevin, Lande, & Mace, 2010). Local adaptation is defined as a population having higher fitness in its native environment than any other environment (Blanquart et al., 2013). If there is environmental heterogeneity, populations can persist when a phenotype has high fitness and is selected for in the environment. If these phenotypes are heritable, this persistence can result in populations becoming locally adapted to the environment by the mean phenotype in the population shifting to an adaptive peak. However, there are genetic mechanisms that inhibit populations ability to shift their phenotypes to locally adapt such as gene flow (Slatkin, 1987; Urban, 2011).

Gene flow can limit local adaptation by opposing natural selection if the individuals dispersing are maladapted to the environment (Figure 1). For local adaptation to occur there has to be a balance between gene flow and selection in a population (Kawecki & Ebert, 2004). If migration is high, then gene flow could swamp the population’s gene pool with alleles that do not have a high fitness in that particular environment therefore hindering local adaptation. If migration is low and the population has low genetic variability, then gene flow could increase the variation possibly leading to higher fitness in that particular environment and in turn result in local adaptation (Hendry & Taylor, 2004; Lenormand, 2002; Nosil & Crespi, 2004). If local adaptation persists despite gene flow, selection is strong enough to overcome gene flow due to environmental factors (Garant, Forde, & Hendry, 2007). This balance has been observed and documented in many systems with organisms like birds (Smith, Wayne, Girman, & Bruford, 1997), fishes (Hendry & Taylor, 2004; Hendry et al., 2002), plants (Santon & Galen, 2002), reptiles (King & Lawson, 2006), amphibians (Storfer & Sih, 2006), and insects (Nosil & Crespi, 2004). Specifically, a study using sticklebacks (Hendry & Taylor, 2004) determined with high gene flow between two environments the morphology of the organisms did not show much divergence. Morphology can be a way to determine phenotypic differences that have risen due to adaptation, but plasticity is another route which is rarely considered in the studies listed above.

Plasticity, divergent selection, and gene flow contribute to population divergence but plasticity is the only non-genetic response to the environment of these (Crispo, 2008). Phenotypic plasticity is the ability for a given genotype to express different phenotypes in response to the environment. Plasticity is beneficial because it increases the chance of an organism finding an environment to match when more than one phenotype can be expressed (Figure 1). However, plasticity is potentially costly to an organism’s fitness. This strategy has energetic, production, information acquisition and genetic costs to name a few (DeWitt, Sih, & Wilson, 1998). Moderate levels of plasticity are thought to facilitate genetic evolution, by allowing initially maladapted populations to persist, but plasticity also restricts local adaptation because it can shield maladapted individuals’ genotypes from selection (Price, Qvarnström, & Irwin, 2003). Plasticity is hard to detect in the wild, but in a laboratory setting plasticity can be controlled for through common garden experiments.

Common garden experiments are a way to control environmental effects and is useful to test for local adaptation, divergent selection and plasticity, along with other methods like reciprocal transplant experiments (Savolainen, Lascoux, & Merilä, 2013). Common garden transplants are when all organisms are reared under the same conditions eliminating environmental effects, and individuals are then transplanted into different habitats. A reciprocal transplant consists of individuals from local habitats being placed into non-local habitats and recording a measure of fitness of both local and non-local individuals in both habitats (Kawecki & Ebert, 2004; Siepielski, Nemirov, Cattivera, & Nickerson, 2016). If local adaptation is occurring, then local individuals would have higher fitness in their local habitat than individuals from the non-local habitat (Figure 2). The common garden transplant allows for investigation of plasticity by controlling the environmental x genotype aspect. This is done by transplanting individuals into different environments and looking at the fitness differences, in this case growth rates.

Stream bluets, *Enallagma exsulans*, are an ideal organism to use while investigating these ideas because this species is found in stream and lake environments in close proximity with no geographic barrier. This close proximity allows for potential gene flow between the two populations and is a good system to investigate local adaptation and the influence of selection, gene flow and plasticity on population divergences. Setups like this have been used before with sticklebacks in stream and lake populations that are found in close proximity on a fine spatial scale (Bolnick et al., 2009; Hendry & Taylor, 2004; Hendry et al., 2002). It has been shown that divergence is still possible between microgeographic sites (Richardson, Urban, Bolnick, & Skelly, 2014).

In order to examine if divergence and local adaptation can occur with no obvious geographic barriers, I set up a reciprocal transplant experiment between lake and stream environments. Each site had replicates of individuals from the native and non-native sites. In addition to the reciprocal transplant with wild caught individuals, to examine if plasticity was occurring also, I reared individuals from both lake and streams in a common garden to complete a second transplant experiment. However, due to severe storms damaging the experiments I was unable to attain any results. I expected to find native individuals perform better than non-native individuals in each site for the wild caught reciprocal transplant, supporting local adaptation. For the common garden transplant, I expected if there is local adaptation for there to be the same patterns as the wild caught transplant, with natives out performing non-natives. If there is phenotypic plasticity I would expect consistent fitness across both environments for natives and non-natives.

METHODS

Study Organism and Study Site

E. exsulans, found in streams and lakes, are an ideal organism for this study because of their ability to be reared in a common garden from eggs to nymphs for use. The larvae are also easily collected from streams and lakes in the area for use with wild caught reciprocal transplant experiments. This study was completed at Lake Fayetteville (36.1375°N, 94.1341°W) and the out-flow stream Clear Creek (36.1327°N, 94.1455°W), on the west side of the lake. The 200-acre lake is in the middle of Lake Fayetteville Park surrounded by a mixed conifer forest as a barrier between the lake and the city. The lake has submerged vegetation in the littoral zone consisting of *Justicia americana*, *Ceratophyllum demersum*, and *Najas minor* for damselfly larvae to perch. The stream also has mixed conifer forest corridors as a barrier for the stream. At the outflow point of the stream, the water is slowing moving with depth and vegetation comparable to the lake. Vegetation is found at the banks of the stream with root banks where invertebrates like damselflies and dragonflies were found. Other segments of the stream are shallow and fast moving with less root banks, rocky stream beds, and no vegetation except at the stream banks.

Reciprocal Transplant

To examine if populations locally adapted to lake and stream environments, I completed a reciprocal transplant experiment. Damselfly larvae were collected at Lake Fayetteville and the outflow stream Clear Creek using a D-frame dip net (28-cm net opening, 1 x 1 mm mesh) in November 2018, once larvae were large enough to ID and of a sufficient size they could not escape experimental enclosures. Larvae were stored in an environmental chamber, on a controlled temperature (16C) and light cycle (8:16), until a sufficient number of larvae were collected from the sites, after which time individuals were randomly assigned to field cages. Cages (44.45 cm x 16.51 cm x 16.51 cm) made of PVC pipe and mosquito netting (1-mm mesh opening) were placed into the lake and stream a week before the experiment started to allow zooplankton and other damselfly prey to colonize the cage and reduce cannibalism. Cages were completely submerged at each site in the littoral zone and filled with macrophytes found near the larval collection sites. This allowed the cages to mimic the natural environment the larvae were collected from, with their common prey and macrophytes present.

Each site, lake and stream, had a set of 4 replicates of native larvae from each site and a set of 4 replicates of non-native larvae from the other site. An initial sample of larvae was collected at the stream and lake for growth rate measurements, which is done by comparing this initial sample larvae head widths to the heath widths of the larvae used in the experiment after completion. The experiment ran three weeks from mid-November through the beginning of December, which is a sufficient amount of time to detect growth rates in the field (McPeek, 1998; Siepielski, DiBattista, Evans, & Carlson, 2011; Siepielski, Hung, Bein, & Mcpeek, 2010). However, the cages were damaged in a storm in both the lake and stream and there were not enough larvae recovered to gather any results. Using the initial sample and the resulting larvae from the experiment, growth rates would have been estimated, as a component of fitness, by using head widths. Growth rates are an important measure of fitness because it shows the organisms ability to utilize environmental resources. To estimate growth rates, I would have used the mean cage values and mean initial sample values of each body metric to estimate growth rates of native and non-native individuals in each environment using the equation growth rates=(Ln(Mt) – ln(Mo))/tm which assumes a model of $M_t = M_o e^{rt}$, where Mt and Mo, are the morphological trait values at time t and initially, respectively, and is independent of initial size. Cage means were used because individual growth rates cannot be measured with damselflies because of molting and there is no way to mark or track each individual once placed into a cage.

The expected result of a local adaptation experiment would be a significant interaction effect between source population and experimental location. If local adaptation is present, then native individuals should have higher growth rates in their local environment compared to non-native individuals. To test for this effect, I would have used a two-way analysis of variance, including terms for experimental location, source of individuals, and the interaction term.

Common Garden Rearing

To control for the potential effect of phenotypic plasticity, I reared larvae from streams and lakes in a common garden over the summer and fall of 2018 then used a similar as above where I transplanted them into the lake and stream environments. The results of this test would have been compared to the wild caught reciprocal transplant results to examine if phenotypic plasticity played a role in shaping the performance of larval damselflies in each environment.

Adult *E. exsulans* mating pairs in copulation were collected at the stream and lake site using an aerial net over the months of May and June. The pairs were placed into 2-ounce clear cups with 2cm of water in the bottom of the cup, a piece of wet coffee filter paper placed against the side of the cup for the eggs to be laid, and another piece of coffee paper with holes over the top of the cup. The coffee paper was checked for eggs a few days after collection or when the adults died. Once eggs were laid, the portions of the paper containing the eggs were removed and the cup with filled with lake or stream water and the eggs were submerged. The cups remained in the lab under room temperature and natural light conditions until larvae hatched. Then cups were placed in the environmental chamber that controlled temperature at 24C and light availability at 16 hours of light each day to mimic natural conditions at this time. Temperatures were lowered over the course of the summer and fall to 16C for the larvae to be acclimated to the conditions of the streams and lake in November for the transplant. Once hatched, larvae were separated individually into 60mL clear cups with water from their respective site. The larvae were fed a mixture of dechlorinated water and brine shrimp daily. Except during the last month before being placed into cages the larvae were fed zooplankton from a lab-maintained stock containing mostly daphnia and ostracods. This helped acclimate the larvae to the prey that would be found in the cages at the lakes and stream.

As above, a week before the start of the transplant, cages were placed into the lake and stream environments to allow natural prey to colonize for when the larvae are placed. Also, before the transplant, a subset of 20 lake and stream larvae were preserved in 70% ethanol as an initial sample for future growth rate estimates and geometric morphometric analyses.

Common Garden Transplant

The same set up and process for cages in the wild caught transplant were used in the common garden transplant. There were eight cages at each site; four of the cages had 20 individuals each from the native site and the other four had 20 individuals each from the foreign site. After 21 days the cages would have been removed, and the larvae would have been brought back into the lab and placed in 70% ethanol. The larvae from the experiment along with the initial sample would have been weighed and head widths, wing pad length and body length would have been measured for each individual to estimate per capita growth rates, all as described as above.

The expected result would be there is no significance fitness differences between native individuals and non-native individuals in an environment in a two-way analysis of variance. If plasticity is present, then individuals would have no significant fitness difference in their native or nonnative environment. The individuals would respond to the environment placed into.

RESULTS

This transplant was attempted two times during the 2017 and 2018 field seasons. Neither attempt was successful. The first attempt resulted in contaminated cages with other larvae found at the end of the experiment that were not part of the transplant. The cause of this was unknown. The second attempt failed due to weather. The cages and setup used are good for the lake environment. The setup for the stream environment needed to be improved even though the cages were tied to cement blocks and not in a relatively high flow area in the stream. Stream cages were destroyed and found after being pushed downstream from a storm. Larvae were recovered from some cages but there was no way to determine if the larvae were ones used in the experiment or ones that had entered the cages after the mosquito netting was ripped. Lake cages had been stressed and pushed around during the storm resulting in the mosquito netting ripping and losing larvae.

Despite the field experiment failing, I was able to use larvae from my common garden experiment for geometric morphometric analyses. I was also able to do a small scale mesocosm study in the lab of my reciprocal transplant study with common garden individuals. The results of these tests can be found in Chapter 2.

DISCUSSION

Although there are no results, the reciprocal and common garden transplants would have allowed for the investigation of local adaptation in stream and lake populations of *E. exsulans*. Local adaptation is important because of the ability to help populations persist despite environmental change (Chevin et al., 2010). If growth rates could have been obtained, this study had the potential to show if native larvae were locally adapted and more fit than the non-native larvae in the environment despite potential high gene flow due to close proximity of the lake and stream.

It is possible there are high amounts of gene flow between the lake and stream sites, and gene flow can either swamp selection or help promote genetic diversity fueling local adaptation (Garant et al., 2007). If there are high amounts of gene flow, but local adaptation is present, then we can assume selection was strong enough to overcome gene flow. But if gene flow is high and there is no local adaptation, then gene flow could be introducing too much variability and not allow the population to adapt to the environment. Another possibility is that the population has very low genetic diversity and gene flow is introducing more diversity, but the population is still not locally adapted. There has to be balance between selection and gene flow for a population to evolve higher fitness in their local environment than other phenotypes introduced (Lenormand, 2002). This balance between gene flow, selection and adaptation has been seen in previous studies similar to the one I attempted. Nosil and Crespi (2004) found evidence for divergent selection based on estimates of trait divergence with species of walking stick insects. This selection was driven by different environmental conditions; in this case populations diverged by which host plant the walking sticks were living on in order to avoid predation. Another study used stream and lake stickle backs and found habitat related divergent selection has influenced morphology of stream and lake sticklebacks resulting in diverged populations (Berner, Adams, Grandchamp, & Hendry, 2008). Bolnick et al. (2009) used parapatric populations of stream and lake sticklebacks to explore the balance of migration and selection in relation to adaptive divergence. Their findings showed most individuals returned to their native habitat but the ones that did not return were found to be more morphologically similar to the non-native type. This habitat preference was phenotypically dependent therefore facilitating divergence and creating a correlation between the genotype of those organisms and the preferred environment. In each of these studies, the contrasting environments were all within close proximity and no obvious geographic barriers to dispersal were present.

Gene flow is usually seen as hindering local adaptation, but it is possible that individuals are not randomly dispersing but rather choosing to stay in an environment they are best fit in after sampling other environments (Edelaar, Siepielski, & Clobert, 2008). This process called ‘matching habitat choice’ could promote population divergence with no *in-situ* local adaptation. For example, if individuals choose an environment to stay in and are the best fit due to phenotype x environment this could lead to a genetic x environmental correlation (assuming the phenotypes had a genetic basis) and eventually facilitate greater local adaptation or divergence. The individuals dispersing are potentially able to choose their environment by sampling or visiting multiple environments to choose where they will be best fit but this is costly. Therefore, individuals settle in any environment available while in other cases, individuals may be able to disperse and be fit in any environment visited making them phenotypically plastic, which is also costly (DeWitt et al., 1998). Plasticity is another mechanism that can promote or hinder local adaptation and divergence. If an organism is plastic, there is a better chance of finding an environment to survive. These plastic organisms have the genotype that allows for a phenotype that can survive in multiple environments. When there are various genotypes in an environment, the phenotypes that are expressed have different levels of fitness associated with them. Selection favors phenotypes fit in the environment changing the genotypic frequency in the population, meaning plastic organisms can be selected for by their phenotype expressed leading to adaptive evolution. This is important when environments shift for there to be plastic organisms to express different phenotypes for populations or even species to survive.

Once this initial study is completed there are further studies and improvements that can be done. Adaptive divergence has been studied using reciprocal transplants for local adaptation, but also frequency manipulations for investigating frequency-dependent selection. For a population to become locally adapted there are tradeoffs making them less adapted in other environments and the strength of competition could influence local adaptation and vice versa (Peterson, Rice, & Sexton, 2013; Siepielski et al., 2016). Damselflies have been shown to exhibit density dependent competition (McPeek, 1998; Siepielski et al., 2010; Siepielski, Mertens, Wilkinson, & McPeek, 2011) so this study system could accomplish the goal of determining if local adaptation and interspecific competition are coupled. A previous study using sticklebacks was the first to combine these two experimental approaches and concurrently investigate local adaptation and density dependent competition (Bolnick & Stutz, 2017). This experimental design could show there is a relationship between evolutionary and ecological processes at the population level with damselflies as the study showed with sticklebacks.

If results were obtained, this study would have determined if there is divergence between stream and lake *E. exsulans* despite there being gene flow between the two populations. It remains an important question and can be expanded on in the future after initial findings. Overall population divergence and adaptation with changing environments has important implications for maintaining populations and diversity.

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FIGURES

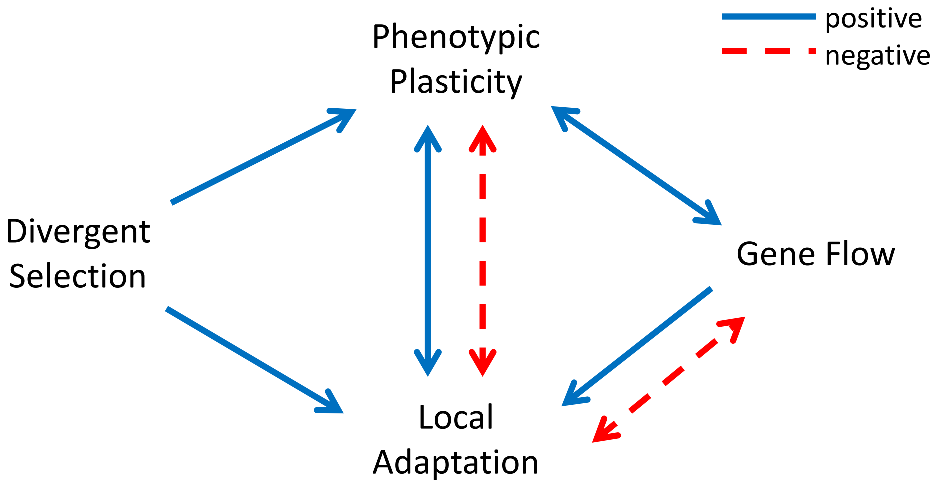

Figure 1. Diagram showing relationships between factors influencing a population. (Based on Crispo 2008 figure 1)

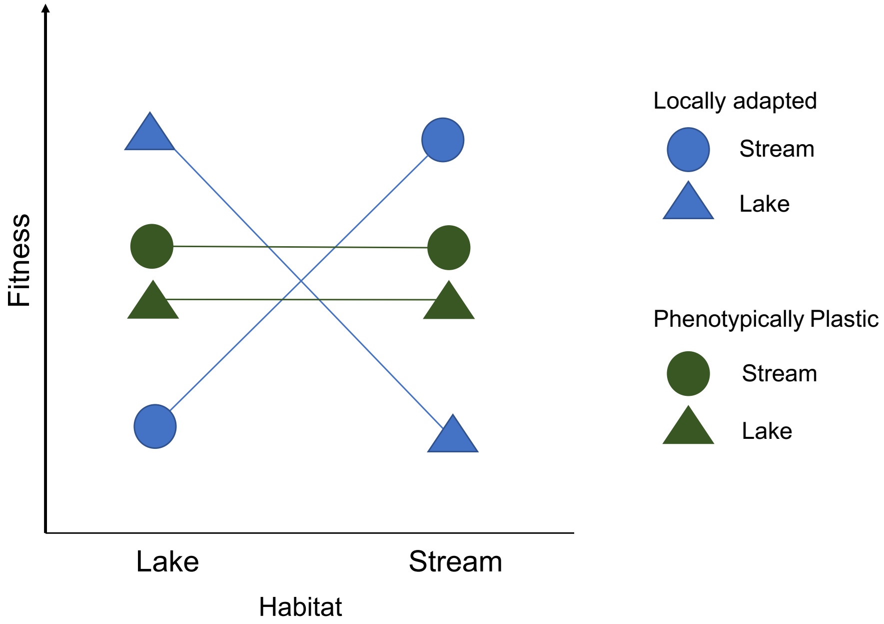

Figure 2. Locally adapted individuals (blue shapes) have a higher fitness in their local environment than foreign environments. Locally adapted individuals have higher fitness than non-local individuals entering them environment. Phenotypically plastic individuals (green shapes) show no fitness differences between environments. These individuals perform consistently based on their ability to express more than one phenotype as a response to the present environment.

CHAPTER 2: MORPHOLOGICAL DIVERGENCE OF STREAM AND LAKE *ENALLAGMA EXSULANS*

ABSTRACT

Environmental heterogeneity can promote divergent selection, which could produce differences in the morphology and behavior of populations in different environments. It is important for populations to be able to adapt to changing environments and starts with individuals that are able to express different phenotypes as a response to the environment present. These phenotypic changes can be morphological or behavioral for the organism to survive in different environments. Morphological or behavioral characteristics could also be the result of local adaptation to an environment. Local adaptation promoting different morphology and behavior as a response to the environment could lead to population divergence. This study aims to determine if there is behavioral or morphological divergence between stream and lake *Enallagma exsulans*, by using behavioral assays and a geometric morphometric approach, respectively. I analyzed samples of wild caught *E. exsulans* from 22 lake and stream sites in the Northwest Arkansas region and from common garden reared stream and lake *E. exsulans* from one stream and lake pair. I found significant shape differences between wild caught stream and lake *E. exsulans* for body and lamellae shape. For the common garden reared stream and lake *E. exsulans* I found no significant body or lamellae shape differences. These results suggest there is not a genetic basis for the divergence seen in the wild caught stream and lake damselflies. A small scale mesocosm reciprocal transplant with common garden stream and lake individuals transplanted into lake conditions and stream conditions showed no significant growth differences between condition or lake and stream origins. Common garden reared individuals were also used in behavioral assays, but no divergence was found between the populations.

INTRODUCTION

Local adaptation can produce phenotypic differences between populations like differences in morphology or behavior. Phenotypic differences between populations in the wild is usually the result of the relationship between gene flow and selection in the population as discussed in Chapter 1. In some wild populations of *Gasterosteus aculeatus*, phenotypic differences between stream and lake individuals have been found as evidence for divergent selection (Hendry, Taylor, & McPhail, 2002). While in other populations, the phenotypic differences between stream and lake individuals is low because of high gene flow (Berner et al., 2008; Hendry & Taylor, 2004; Moore, Gow, Taylor, & Hendry, 2007; Moore & Hendry, 2005). Phenotypic differences could also be the results of a non-genetic mechanism like plasticity (Mccairns & Bernatchez, 2012; Rajkov, Weber, Salzburger, & Egger, 2018; Sharpe et al., 2008; Taugbøl et al., 2014). If phenotypic divergence in populations is genetically based, then populations reared in a common garden would show significant differences in phenotypes because the environmental effect has been removed (Sharpe et al., 2008). If there is no significant difference, this would imply an environmental effect. The phenotype of wild caught individuals would need to be analyzed to determine the importance of environmental effects. This analysis could be done using morphology. If there is a difference in morphology of wild caught stream and lake individuals, morphometric analyses could be a way to detect divergent selection between lake and stream populations.

Geometric morphometrics aims to assess shape and shape variation to investigate morphological change (Cooke & Terhune, 2015; Lele & Bookstein, 2006). This process was derived from the traditional morphometric analysis which consisted of linear measurements and ratios. Traditional morphometrics would just show a shape change, but geometric morphometric analyses can show how the shape has changed relative to the other structures and their position (Adams, Rohlf, & Slice, 2004; James Rohlf & Marcus, 1993). In this study, I used geometric morphometric analyses to investigate if there are shape differences in stream and lake individuals potentially due to divergent selection (Taugbøl et al., 2014).

Morphological variation combined with behavior plays a role in functional differences in each population. These morphological differences influence organisms' ability to interact with the environment (Mark A. McPeek, 2006). For example, damselfly larvae, propel themselves by swinging their abdomens side to side using the three caudal lamellae as 'paddles'. (M A McPeek, 1997). Because of their functional importance, lamellae can show morphological differences in lamellae between stream and lake individuals potentially reflecting divergent selection based on environmental factors. Variation in shape and size of abdomen and lamellae. Wider and longer lamellae would provide more propulsion and be advantageous in fast flowing water of stream environments as compared to lake environments. Indeed, a study found the phenotypic differences between populations was most likely due to local adaptation to stream and lake environments, which indicates strong divergent selection (Berner et al., 2008). Another study found that the body shape of sticklebacks in a stream environment was more streamlined, reflecting adaptation for better swimming ability in flowing water (Sharpe et al., 2008). If there is morphological variation between stream and lake populations of damselflies, then geometric morphometric analysis will help detect these differences.

Morphological variation between populations in different environments can demonstrate local adaptation to the present environment. A common garden approach eliminates the environmental effects for the genetic basis of phenotypes expressed. Previous work with sticklebacks in a common garden showed phenotypic differences without environmental effects suggesting these phenotypic differences have a genetic basis (Hendry et al., 2002). If there are no phenotypic differences between populations raised under identical conditions, plasticity is likely the underlying mechanism that aides these organisms to survive in different environments. Plasticity is the ability of an organism to express more than one phenotype depending on the environment present. A reciprocal transplant of stream and lake individuals into lake and stream conditions would test if these individuals from stream and lake populations are locally adapted to their native environments or exhibit phenotypically plastic in response to the current environment. If local adaptation is present, native individuals would outperform non-native individuals in the native condition. If individuals exhibit plasticity, native individuals would perform consistently in the native and non-native environment. Since this original field plan failed (see Chapter 1), this idea to test for local adaptation was tested on a smaller scale with mesocosms, in the lab using damselflies.

Stream bluets, *Enallagma exsulans*, are a good system for this study because they occur in stream and lakes across the region. The stream and lake collection sites from Lake Fayetteville and Clear Creek are geographically close enough for gene flow to be possible. The individuals reared in a common garden experiments originated from mating adult pairs caught at these lake and stream sites. All other sites *E. exsulans* were collected from geographically isolated sites.

I used larvae from multiple stream and lake populations across the area for geometric morphometric analysis. I expected there to be divergence in the morphology between stream and lake individuals. I expected the stream *E. exsulans* to have a more stream-lined body and wider, more paddle like lamellae than the lake *E. exsulans*. For individuals raised under common garden conditions, I expected shape to be similar to the wild caught larvae indicating there is a genetic basis of the observed morphological variation. In contrast, lack of differences would suggest that the environmental (i.e., stream or lake habitat) shapes morphology or phenotypes of those individuals. In mesocosms, individuals with a genetic basis for local adaptation, I expected individuals to perform better in their native environment than in the non-native one. Plasticity could be present if individuals perform consistently in both environments showing they can match their phenotype to the current environment. Behaviorally, I predicted stream *E. exsulans* would demonstrate stronger swimming abilities and attach to substrates quicker in both environments. I expected lake larvae to perform worse than stream larvae due to body and lamellae shape and swimming abilities. Additionally, I predicted it would be easier for stream larvae to perform better in still water than for lake larvae to perform better in flowing water.

METHODS

Geometric Morphometric analyses

Geometric morphometric analyses were completed to quantify shape variation among *E. exsulans* from stream and lake populations. The approach is based on homologous, anatomical landmarks identified on each larvae (Figure 1A and 1B).

Two sources of *E. exsulans* larvae were used: specimens from the two experiments explained above, as well as larval samples stored in 70% ethanol collected during previous surveys of lakes and streams across the region in 2016-2018 (Figure 2). I captured digital images of over 400 individuals. Each larva was placed with the ventral side facing up and each leg extended outwards (Figure 1A). The lateral and median lamellae were removed and laid flat for a separate picture (Figure 1B).

A geometric morphometric analysis was used so that all measurements of each segment landmarked are scaled and individuals of all sizes are comparable. The R package, GeoMorph (Adams and Otárola-Castillo 2012), was used for the statistical analysis of shape data. The process begins by placing all images into the same folder with a .CVS file to classify each image with a number, name, and origin of habitat of the individual (lake or stream). With a geometric morphometrics-based approach, I placed landmarks onto pictures of organisms and manually approved each landmark made. The raw landmark data was recorded as Cartesian (x, y) coordinates into a .tps file in the folder with the images and .CVS classifier file. The raw data cannot show any relationships between landmarks or shape until an appropriate 2D or 3D function is fitted (James Rohlf & Marcus, 1993). Procrustes superimposition removes non-shape variation by overlaying the specimens or pictures and standardizing all specimens (Figure 3). This process centers all specimens, scales them to the same size, and rotates them until all specimens are in the same orientation then shows the differences as residuals (Adams et al., 2004). Procrustes superimposition removes statistical degrees from the data and allows the raw data to become shape data to be analyzed. The variation in shape data can be visualized in a Principles Components Analysis plot by placing each specimen, or group of landmarks, in shape space. The closer two points are, the more similar the shape. On the PCA graph the x-axis is PC1, showing the largest variation in shape, and the y-axis accounts for the next largest PC uncorrelated to the first. If stream and lake individuals in this study are morphologically different, then the plot points for streams would be closely grouped on the graph and the lake points would be closely grouped.

A Generalized Procrustes Analysis (GPA) was the first step in the analysis of the raw coordinate data, which removed any variation not due to shape. The GPA-aligned coordinates were used to complete a Principal Components Analysis. This analysis showed the variation between all the individuals and helped determine if there were morphological differences between lake and stream *E. exsulans*. The PCA plot points were used to determine which individuals are morphologically different. I expected to see all of the stream points clustered together, indicating them to be morphologically similar. An ANOVA was also completed to see if there was an effect of origin on morphology.

Mesocosms

A common garden experiment was set up to test the effect of stream conditions and lake conditions on lake and stream individuals. Pans were set out in the greenhouse to rear larvae under identical conditions. Larvae came from adult mating pairs sampled at a stream and lake sites. The pans for the mesocosms were circular with a piece of PVC pipe in the center so the water would flow in one direction in the stream condition. All of the pans were filled with dechlorinated tap water and flow generators were placed into half of the pans to mimic stream conditions while the other half were still water mimicking lake conditions. The larvae were randomly assigned into 4 groups of 7 individuals each: stream individuals placed in lake conditions and stream conditions, and lake individuals placed in lake conditions and stream conditions. Each pan contained one individual. The larvae were starved for 24 hours before the experiment to clear the digestive tract and wet body mass was measured on a microbalance. To estimate growth rates, I would have used the mean initial masses and mean final masses of each group to estimate growth rates of native and non-native individuals in each environment using the equation growth rates=(Ln(Mt) – ln(Mo))/tm which assumes a model of $M_t = M_o e^{rt}$, where Mt and Mo, are the morphological trait values at time t and initially, respectively, and is independent of initial size. The larvae were placed into the treatments for 4 days, which is sufficient time to detect growth rates by body mass. After 4 days, the larvae were removed and weighed again. Over the 4 days, larvae were fed a lab stock of zooplankton, mostly daphnia and ostracods. A two-way ANOVA was used to determine if there were significant effects of larvae origin or condition on the growth rates of the larvae.

Behavior Trials

Using larvae from the environmental chamber, I completed behavioral assays to assess differences in stream and lake individuals when placed into stream and lake conditions. The pans from the flow tests were used again with dechlorinated water. I set up one pan with still water to mimic lake conditions and another pan with flowing water to mimic a stream. I gathered 45 stream and lake individuals from the common garden rearing and placed each individual in the flowing water for one minute and the still water for one minute so every individual from both origins experienced both conditions. During the one minute, I recorded the number of swims, defined as the number of times an individual swung its abdomen from one side to the other and back to the initial side. I recorded the time the larvae spent floating (making no swimming moves) from the time placed in the water until the first swim move was observed. I also recorded the time the larvae attached to a substrate. A repeated measures ANOVA was used, since each individual was observed twice, once in stream conditions and once in lake conditions. This analysis compared the means of stream and lake origin groups across the 4 measurements (floating time, swimming time, time spent attached and number of swims).

RESULTS

Geometric Morphometric Analyses

For the wild caught larvae, PCA of lamellae shape showed 84.4% of variation was accounted for in PC1 and PC2. Principal components take a large amount of possibly correlated variables and transforms them into a smaller number of uncorrelated variables. The first PC, accounts for as much variation as possible and each succeeding principal component accounts for as much remaining variability as possible. For the wild caught larvae, PCA of body showed, 42.8% of variation was accounted for in PC1 and PC2. For wild caught larvae, there was no clear grouping of lake and stream larvae based on body shape (Figure 4). In contrast, shape variation of lamellae revealed a group of stream larvae to be clearly morphologically more similar to each other than they were to lake populations (Figure 6). For the common garden PCA plots with both body shape and lamellae shape, there was no clear distinction between groups (Figure 5 and 7).

An ANOVA was ran on the Procrustes aligned coordinates from the GPA analysis using the function procD.lm using the distances among specimens for analysis (Goodall, 2018). The Procrustes ANOVA revealed significant differences between habitats for wild caught body shape (F=4.6104, p=0.001) and lamellae shape (F=166.75, p=0.001) but not for common garden body or lamellae shape.

Mesocosms

Almost all of the larvae in the small scale mesocosms across all conditions and origins lost body mass over the 4-days. Figure 8 shows a box plot of the growth rate data for the flow trial mesocosms with the median, minimum and maximum growth rates of lake and stream individuals in flowing and still water conditions as well as individual points for the growth rate of each larva. A repeated measures ANOVA was completed since the larvae were measured twice, initially at day 1 and after the trial at day 4. This ANOVA accounted for larva origin, condition, time point, and all combinations of the three (Table 6). Even though there were no significant differences shown from the ANOVA, the lake individuals showed greater variation between individuals in the flowing water and the stream individuals showed greater variation between individuals in the still water (Figure 8).

Behavior Trials

Overall, no significant differences were found for behavior of stream and lake larvae based on origin. There were significant differences based on the condition larvae were placed into. Two-way ANOVAs were completed for each behavior measured: number of swims (Figure 9), time spent swimming (Figure 10), time spent floating (Figure 11), and time spent attached to a substrate (Figure 12). Generally, larvae performed consistently in each condition no matter their origin. I hypothesized stream individuals would attach faster than lake individuals in flowing water because of their ability to hold onto macrophytes and perch while there is flowing water. Stream individuals actually spent more time attached to a substrate in the still lake water condition. Time spent swimming in still water was very low (both average below 10 seconds) because most larvae just sank to the bottom when placed in the water but in the flowing water larvae spent more time swimming.

DISCUSSION

Geometric morphometric analyses of *E. exsulans* from different stream and lake environments across the region show there is morphological divergence between stream and lake types. There were significant differences found in body shape and lamellae shape for wild caught individuals suggesting divergence among populations found in stream and lake environments. The common garden individuals showed no significant differences in body or lamellae shape between stream and lake environments, suggesting this divergence is not genetically based. These differences could be a plastic response to the stream and lake environments (Mccairns & Bernatchez, 2012; Price, Qvarnström, & Irwin, 2003; Scheiner, 2003).

Damselfly adult wings and other insect species wings and larvae have been used for geometric morphometric analyses, but no studies have used damselfly larvae (Lee & Lin, 2012; Outomuro, Adams, & Johansson, 2013). Even with a small amount of wild caught stream individuals relative to lake individuals, in this study, a significant difference in body shape and lamellae shape was detected. Lamellae size difference could indicate variation in swimming ability, and the need for larger, wider lamellae in flowing water. With more morphological studies similar to this, we could determine which structures to focus on that are possibly being selected for in the stream or lake. From this study, it seems the lamellae are one structure to focus on and this structure has a functional purpose (M A McPeek, 1997; Mark A. McPeek, Schrot, & Brown, 1996).

In future studies, a more thorough common garden approach could be applied. If mating pairs could be collected across multiple lake/stream pairs, reared from eggs, and then transplanted into multiple environments this would be a more thorough experimental design. The behavior assays can be advanced as well with recordings of individuals and scoring their behavior and movements based on swimming, foraging, grooming, and walking in the still water and flowing water environments (Ousterhout, Graham, Hasik, Serrano, & Siepielski, 2018). In the small scale mesocosm flow trials, a smaller containment for larvae might have been helpful. The larger containers could have influenced the larvae's ability to obtain prey. Damselflies are sit and wait predators (Siepielski, Wang, & Prince, 2014) therefore in a larger container their chances of coming into contact with prey is lower which could explain why the majority of larvae across both conditions and origins lost body mass. Damselflies are also ambush predators and try to blend in with their environment to not be detected by prey. The mesocosms were dark and the damselflies could have been easily seen and avoided by prey. Another addition would be using wild caught larvae for the mesocosms experiment along with the common garden reared larvae. This could have been set up as a small-scale local adaptation design with reciprocal transplants of lake and stream individuals between lake conditions and stream conditions. It would have been a small-scale lab version of the field design in Chapter 1 for local adaptation, but this would have been a way to track individual growth rates unlike the field design. The behavior trials replicated with wild caught and common garden individuals would be a good way to test if there is behavioral divergence or if it is a plastic response to the environment.

Overall, this study determined there is phenotypic divergence between stream and lake *E. exsulans*. The lack of divergence in the common garden rearing suggests this divergence is not genetically based. However, the phenotypic divergence from the wild caught stream and lake larvae suggests an adaptation to the local environment (Chevin, Lande, & Mace, 2010; Via & Lande, 2006). These differences could be due to other processes like plasticity. If divergent selection is causing morphological differences in stream and lake *E. exsulans* further testing is needed to determine what traits are being selected for in each population. From this study, lamellae shape based on the PCA plot and ANOVA show the biggest difference in stream and lake morphology. Further studies on the lake and stream environments to determine what factors are influencing selection on lamellae shape and size could help determine what’s causing divergence between stream and lake environments.

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TABLES

Table 1. Geographic location of sample sites depicted in Figure 1.

Site Name	County	Site Type	Time of collection	Number of individuals
Bobb Kidd	Washington	Lake	October 2016	1
Bobb Kidd	Washington	Lake	November 2016	6
Booneville	Sebastian	Lake	October 2016	15
Charleston	Franklin	Lake	November 2016	20
Charleston	Franklin	Lake	October 2016	10
Cove	Logan	Lake	October 2016	3
Crystal	Benton	Lake	November 2016	14
Crystal	Benton	Lake	October 2016	4
Darby	Franklin	Lake	November 2016	10
Darby	Franklin	Lake	October 2016	13
Engineer	Franklin	Lake	November 2016	9
Engineer	Franklin	Lake	October 2016	20
Fayetteville	Washington	Lake	November 2016	20
Fayetteville	Washington	Lake	October 2016	15
Fayetteville	Washington	Lake	October 2016	5
Greenwood	Sebastian	Lake	November 2016	3
Greenwood	Sebastian	Lake	October 2016	8
Horsehead	Johnson	Lake	November 2016	18
Horsehead	Johnson	Lake	October 2016	20
Leatherwood	Carrol	Lake	October 2016	7
Leatherwood	Carrol	Lake	September 2016	11
Lee	Washington	Stream	November 2018	5
Lee	Washington	Stream	November 2017	15
Lincoln	Washington	Lake	November 2016	20
Lincoln	Washington	Lake	October 2016	20
Lock and Dam	Sebastian	Lake	November 2016	2
Ozark City	Franklin	Lake	November 2016	10
Ozark City	Franklin	Lake	October 2016	20

Table 1 (cont.). Geographic location of sample sites depicted in Figure 1.

Site Name	County	Site Type	Time of collection	Number of individuals
Paris	Logan	Lake	November 2016	10
Prairie Grove	Washington	Lake	November 2016	6
Prairie Grove	Washington	Lake	October 2016	6
Sequoyah	Washington	Lake	November 2016	7
Sequoyah	Washington	Lake	September 2016	4
Siloam Springs	Benton	Lake	September 2016	14
Town Branch	Washington	Stream	November 2017	4
Wedington	Washington	Lake	November 2016	20
Wedington	Washington	Lake	September 2016	20
Wilson	Washington	Lake	November 2016	9
Wilson	Washington	Lake	October 2016	3
Fayetteville (initial sample)	Washington	Lake	November 2018	16
Fayetteville (initial sample)	Washington	Stream	November 2018	13
Common Garden (Fayetteville)	Washington	Lake	November 2018	20
Common Garden (Fayetteville)	Washington	Stream	November 2018	20

Table 2. ANOVA results comparing lamellae shape between the stream (n=37) and lake (n=415) wild caught *E. exsulans* from the geometric morphometric analysis using 15 landmarks. There was a significant difference in lamellae shape found between stream and lake sites.

Factors	df	SS	Mean Sq	Rsq	F	Z	Pr(>F)
Site	1	8.3517	8.3517	0.27037	166.75	6.0419	0.001
Residuals	450	22.5379	0.0501	0.72963			
Total	451	30.8896					

Table 3. ANOVA results comparing body shape between the stream (n=37) and lake (n=456) wild caught *E. exsulans* from the geometric morphometric analysis with 51 landmarks. There was a significant difference in body shape found between stream and lake sites.

Factors	df	SS	Mean Sq	Rsq	F	Z	Pr(>F)
Site	1	0.1584	0.158422	0.01005	4.6104	3.4854	0.001
Residuals	454	15.6004	0.034362	0.98995			
Total	455	15.7588					

Table 4. ANOVA results comparing body shape between the stream (n=20) and lake (n=20) common garden reared *E. exsulans* from the geometric morphometric analysis with 51 landmarks. There was not a significant difference of body shape between stream and lake common garden larvae.

Factors	df	SS	Mean Sq	Rsq	F	Z	Pr(>F)
Site	1	0.03098	0.030976	0.024	0.9343	0.057723	0.48
Residuals	38	1.25986	0.033154	0.976			
Total	39	1.29084					

Table 5. ANOVA results comparing lamellae shape between the stream (n=17) and lake (n=20) common garden reared *E. exsulans* from the geometric morphometric analysis with 15 landmarks. There was not a significant difference of lamellae shape between stream and lake site common garden larvae.

Factors	df	SS	Mean Sq	Rsq	F	Z	Pr(>F)
Site	1	0.03285	0.032851	0.04595	1.6859	1.1297	0.123
Residuals	35	0.68201	0.019486	0.95405			
Total	36	0.71486					

Table 6. Results from a three-way repeated measures ANOVA comparing growth rates of stream and lake individuals in still and flowing water conditions from the flow trial mesocosms. There were no significant differences found across any factors.

Factors	df	SS	MS	F value	Pr(>F)
Time point	1	2.51	2.5095	1.430	0.238
Origin	1	0.00	0.0012	0.001	0.980
Condition	1	0.48	0.4801	0.274	0.603
Time point + Origin	1	1.02	1.0176	0.580	0.450
Time point + Condition	1	0.88	0.8758	0.499	0.484
Origin + Condition	1	1.46	1.4638	0.834	0.366
Time point + Origin + Condition	1	0.33	0.3290	0.187	0.667

FIGURES

Figure 1A. This image shows an *E. exsulans* larvae used for the geometric morphometric analysis. All larvae were placed in this position for imaging and 51 landmarks (yellow markings) were placed in the same order on each image using the package *GeoMorph*. These landmarks were digitized and converted into x,y coordinates and the raw data was recorded as a .tps file.

FIGURE 1B. This image shows lamellae (left to right: left lateral, right lateral, median) from *E. exsulans* larvae. The lamellae are pictured right side up and the small flat sides of the lamellae attach to the larvae body. These images were digitized separately from the body images but were analyzed as described in Figure 1A.

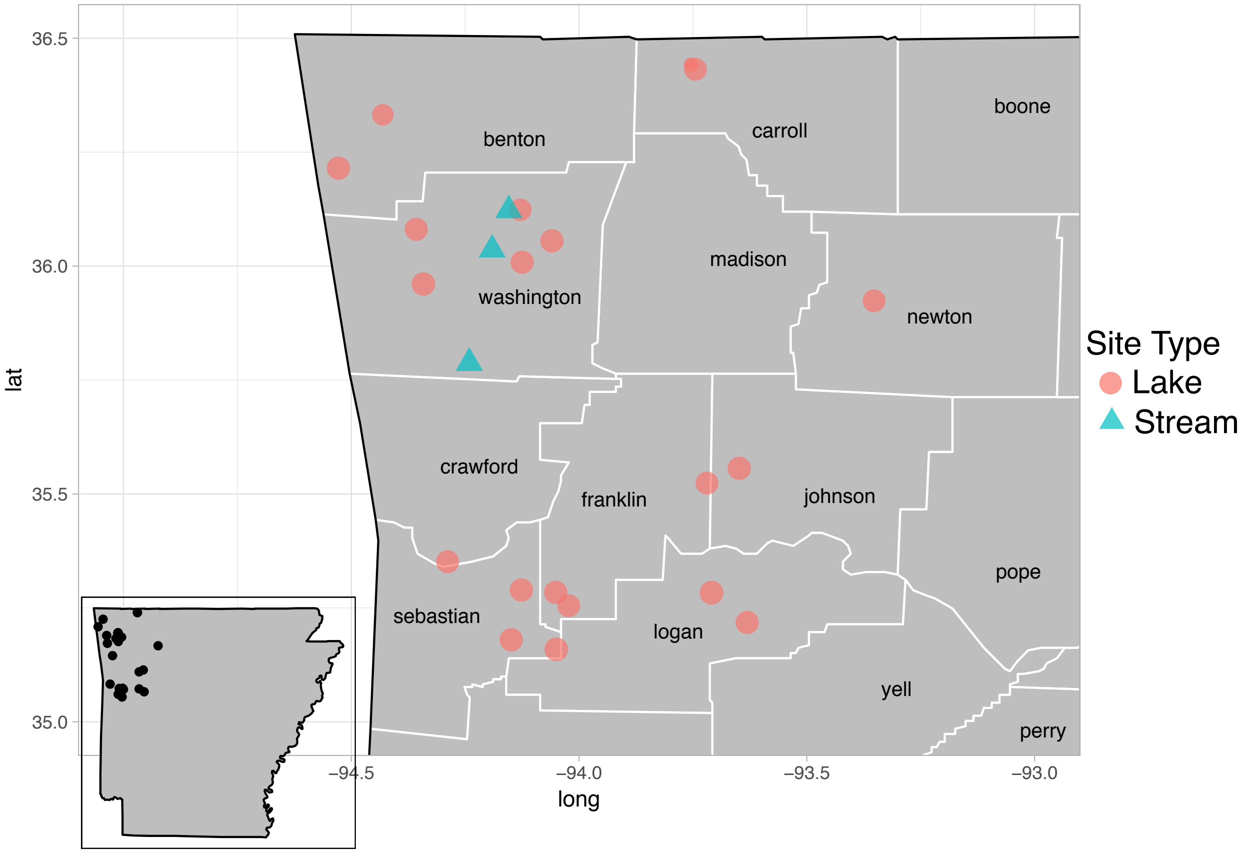

Figure 2. Wild caught *E. exsulans* collection sites from 19 lakes and 3 streams across the Ozark Highlands and River Valley ecoregion. Details on collection sites and sample sizes are provided in Table 1. (Coordinates from *Google Maps*, 2019, maps.google.com)

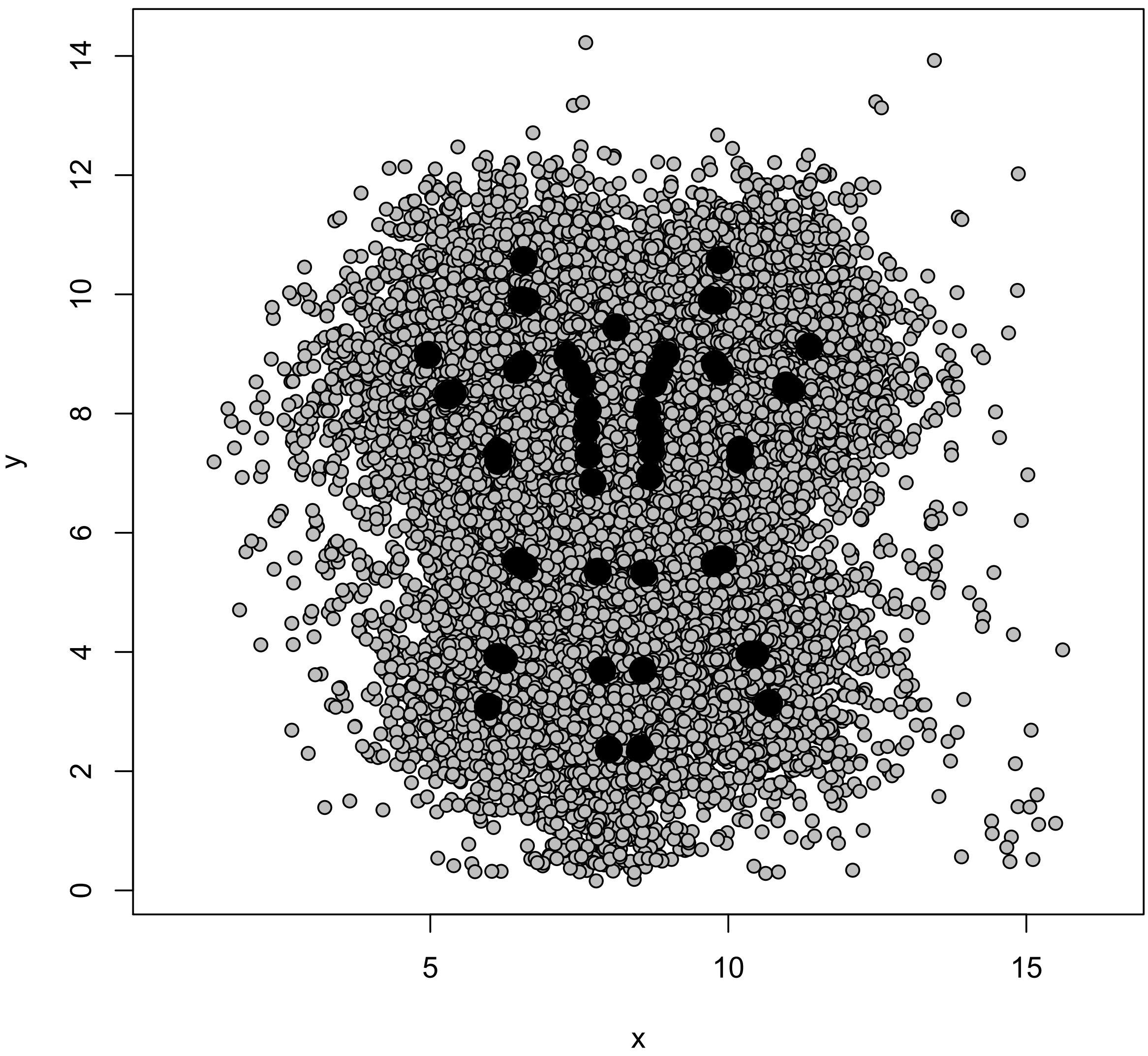

Figure 3. Plot showing all the raw x,y coordinates for each of the 51 landmarks (gray points) on each specimen for body shape from the Procrustes Generalized Analysis. The black points are the centroid region of each of the 51 landmarks.

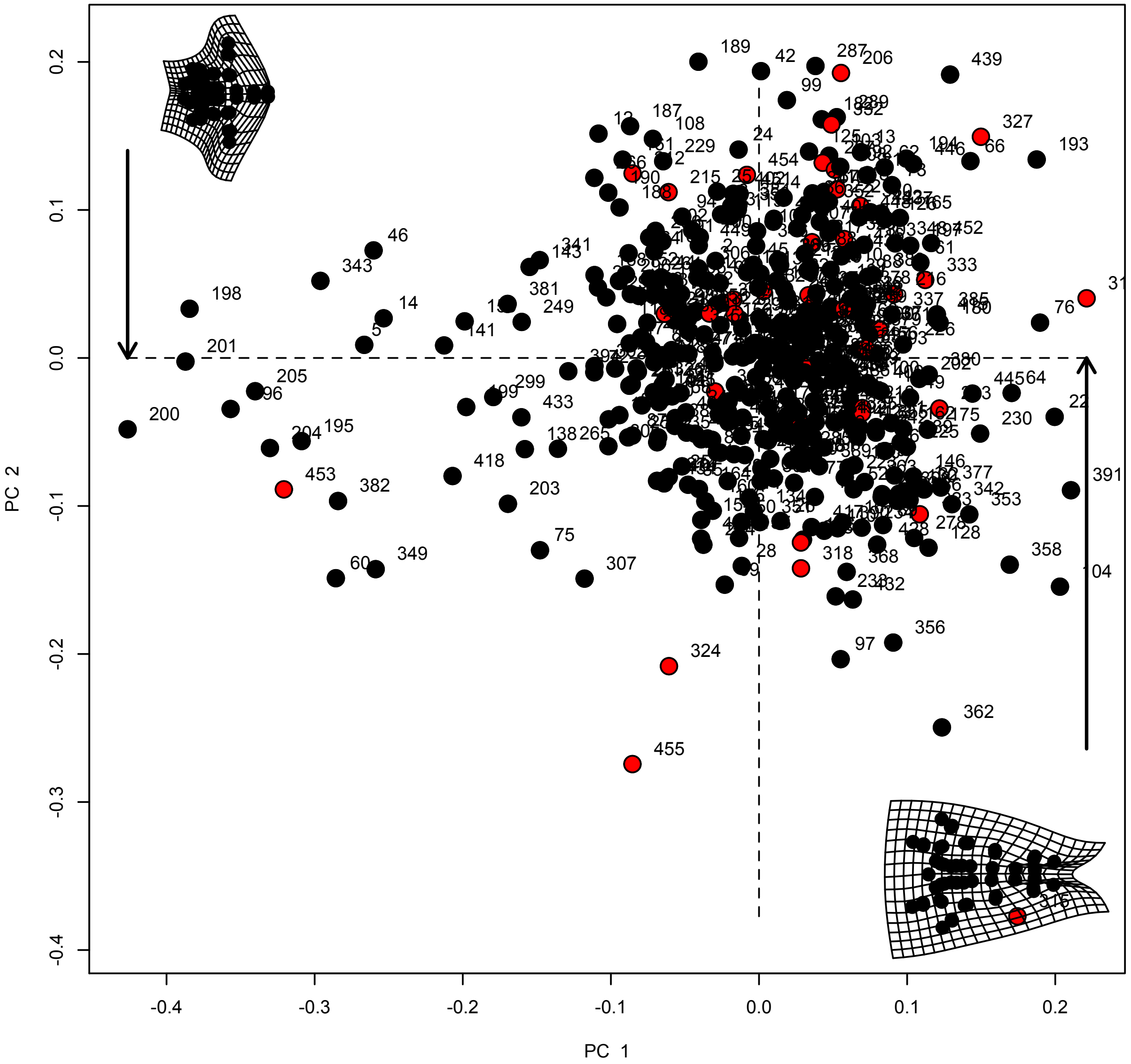

Figure 4. A PCA plot comparing body shape in wild caught samples of *E. exsulans* (n=456) showing PC1 vs. PC2 axes, with PC1 accounting for most of the variation in body size. Each point represents an individual larva, with red points for the stream individuals and black points for the lake individuals. There was a significant difference in stream and lake body shape (F=4.6104, p=0.001).

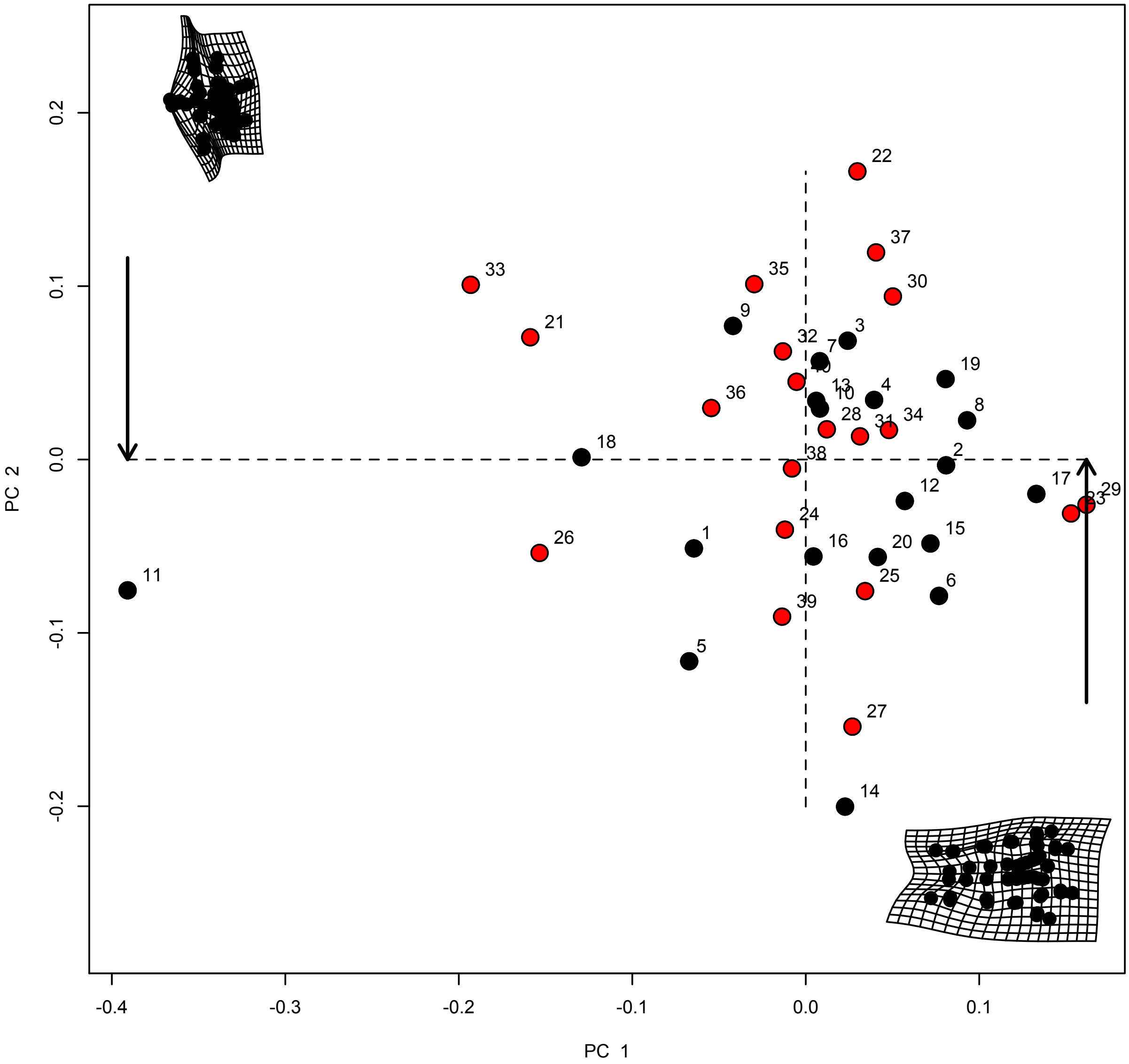

Figure 5. A PCA plot comparing body shape in common garden reared *E. exsulans* (n=40) showing PC1 vs. PC2 axes, with PC1 accounting for most of the variation in body size. Each point represents an individual larva, with red points for the stream individuals and black points for the lake individuals. There was not a significant difference found between common garden stream and lake body shape.

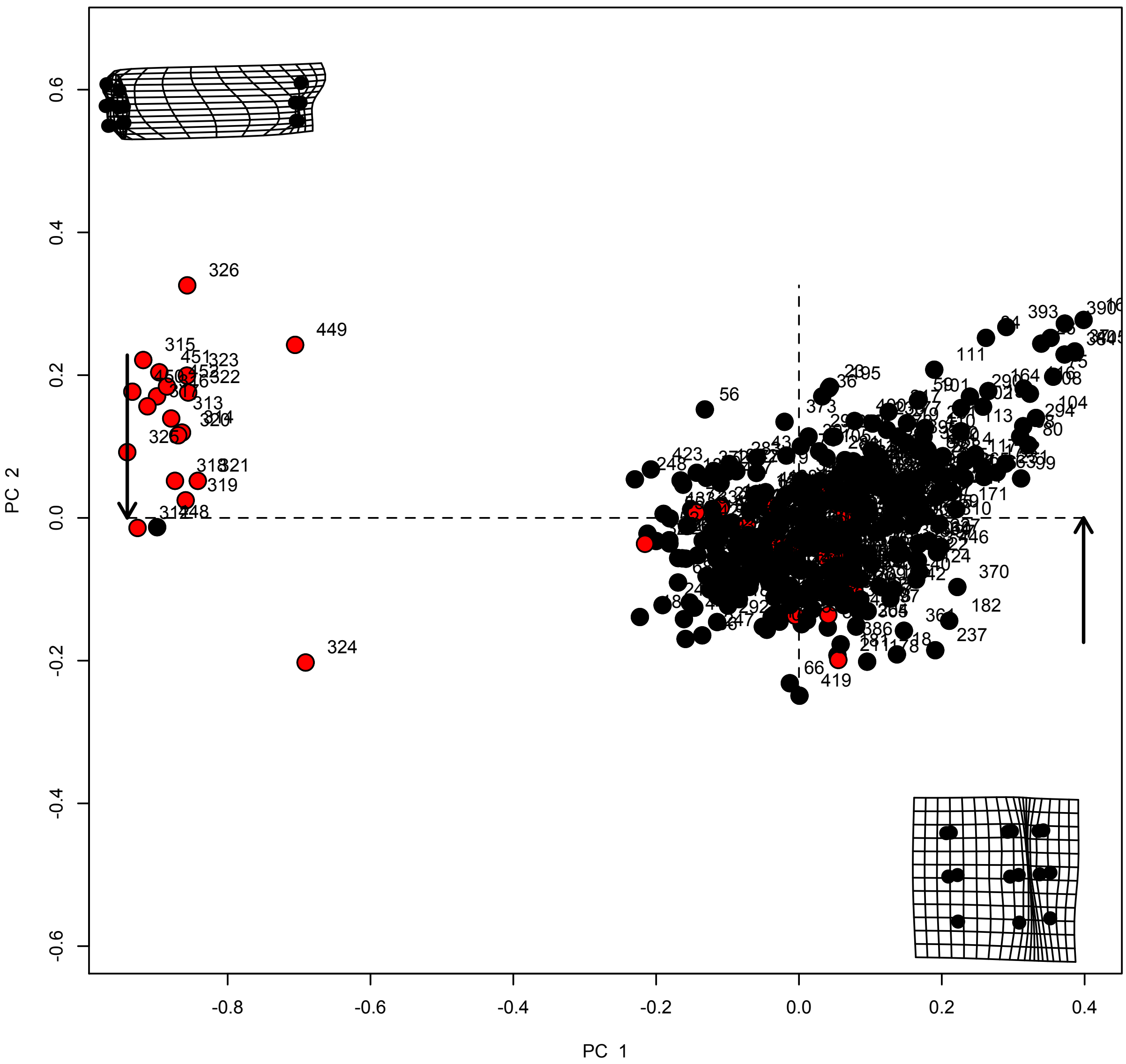

Figure 6. A PCA plot comparing lamellae shape in wild caught samples of *E. exsulans* (n=454) showing PC1 vs. PC2 axes, with PC1 accounting for most of the variation in lamellae size. Each point represents an individual larva, with red points for the stream individuals and black points for the lake individuals. There was a significant difference in stream and lake body shape (F=166.75, p=0.001).

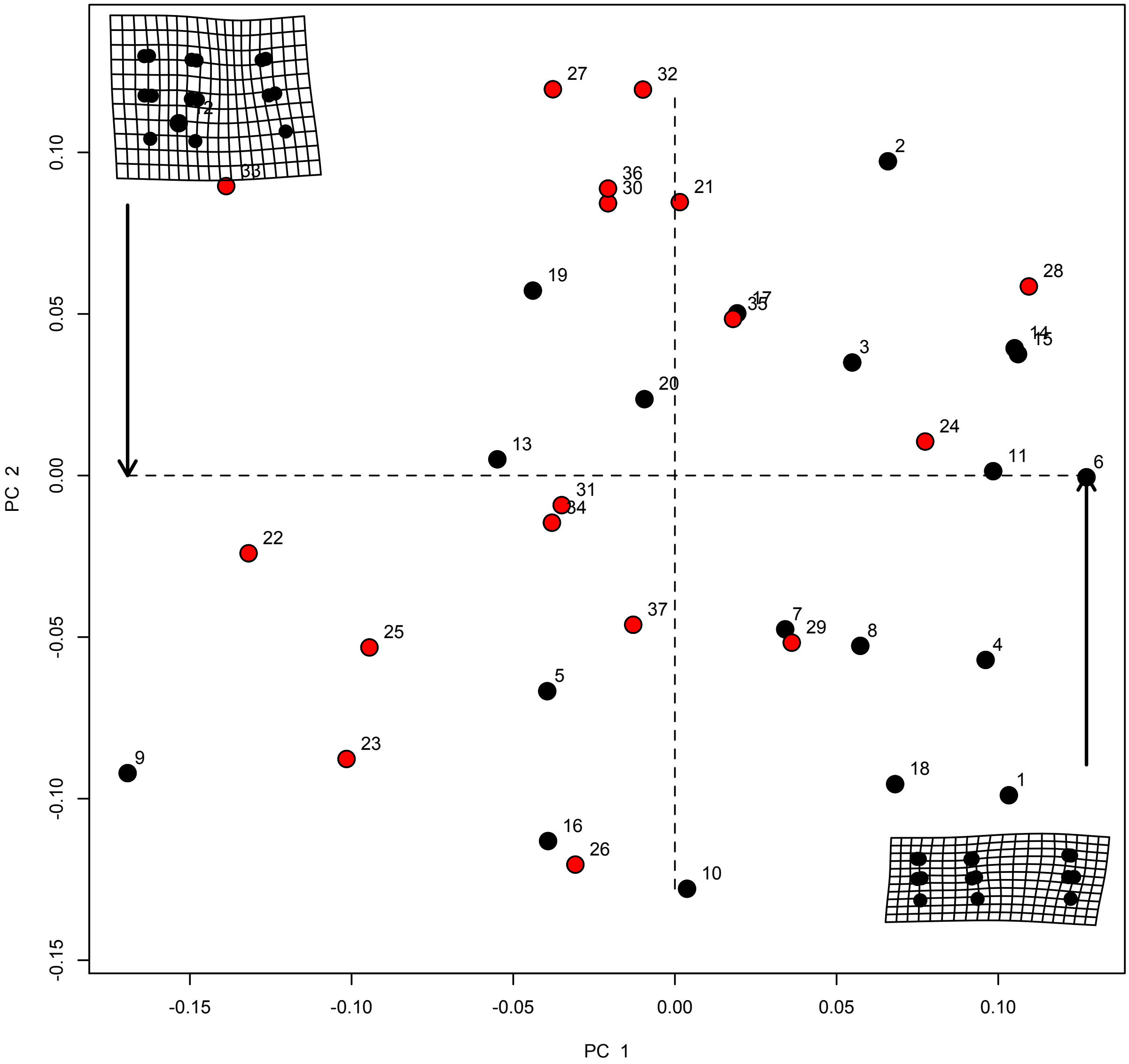

Figure 7. A PCA plot comparing lamellae shape in common garden reared *E. exsulans* (n=37) showing PC1 vs. PC2 axes, with PC1 accounting for most of the variation in body size. Each point represents an individual larva, with red points for the stream individuals and black points for the lake individuals. There was not a significant difference found between stream and lake body shape in common garden groups.

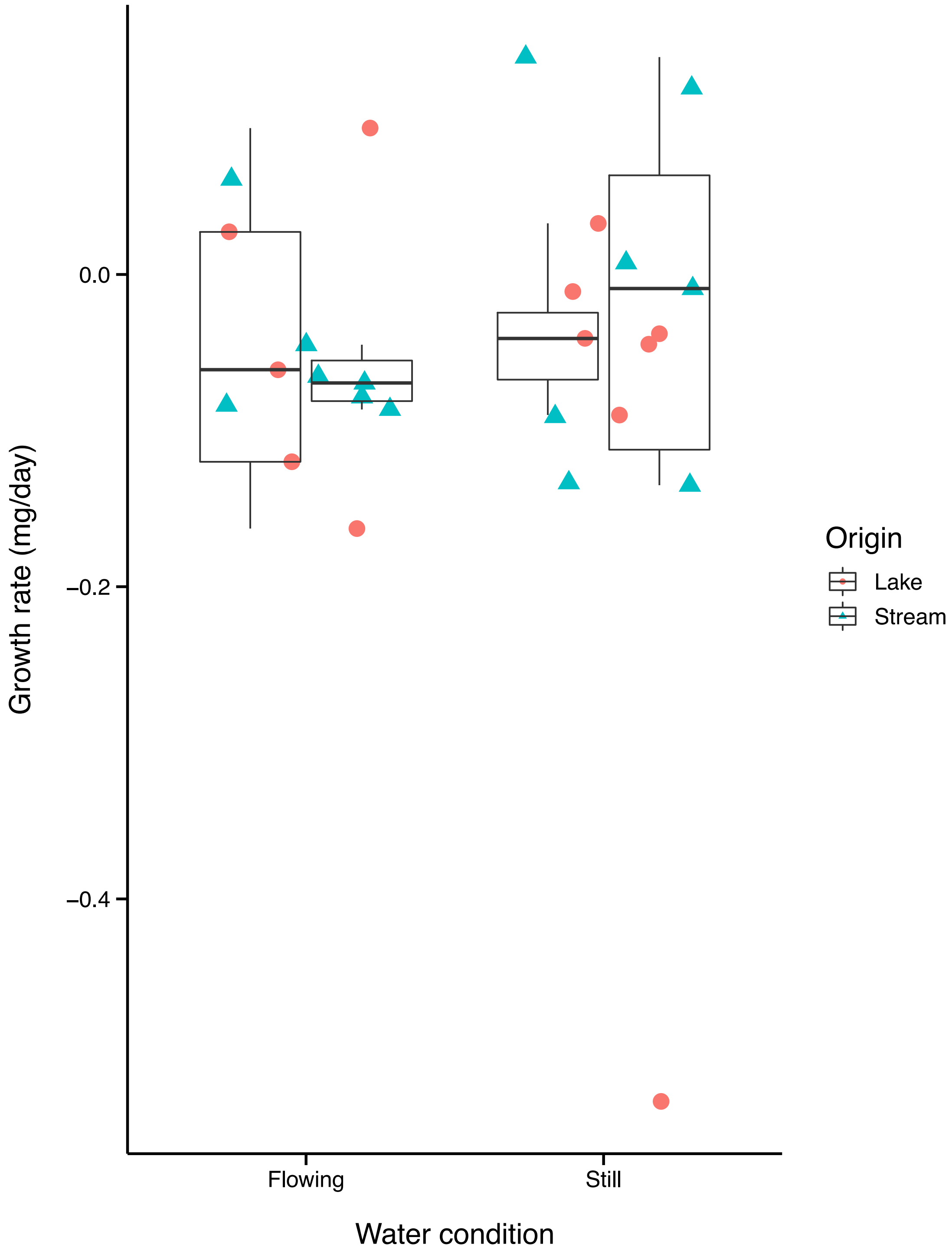

Figure 8. The mesocosm flow trials placed larvae (n=7) from streams and lakes into flowing stream conditions and still lake conditions. This plot shows the median, minimum, and maximum growth rates of each origin placed in each water condition. Each point represents an individual from an origin, based on color. There were no significant effects of origin or condition on larval growth rates.

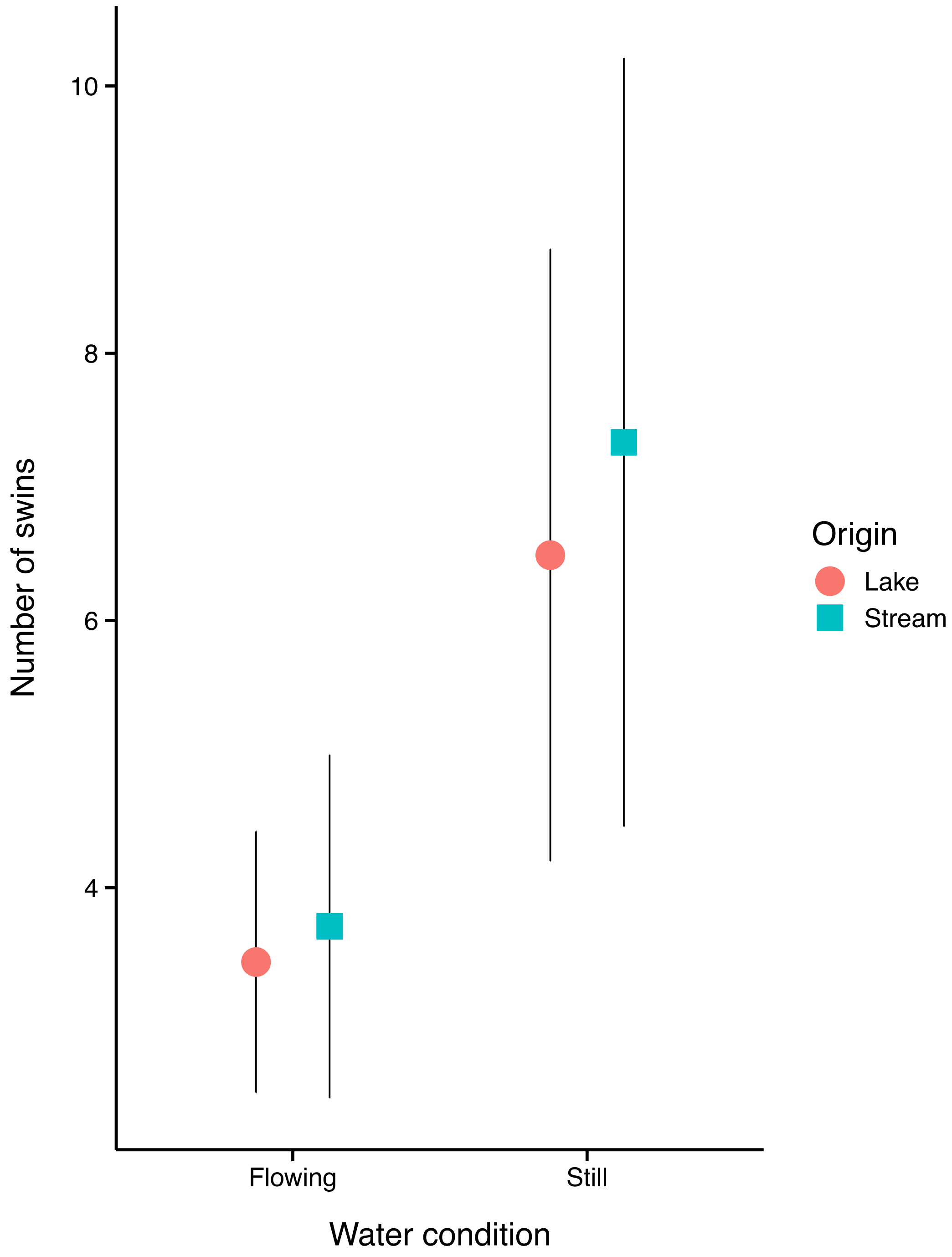

Figure 9. From the behavior assays, this plot shows the means and standard errors of each larvae type placed into each condition. Overall, larvae made more swim moves in the still water condition regardless of origin. There were significant differences found for condition (F=2.759, P=0.0985).

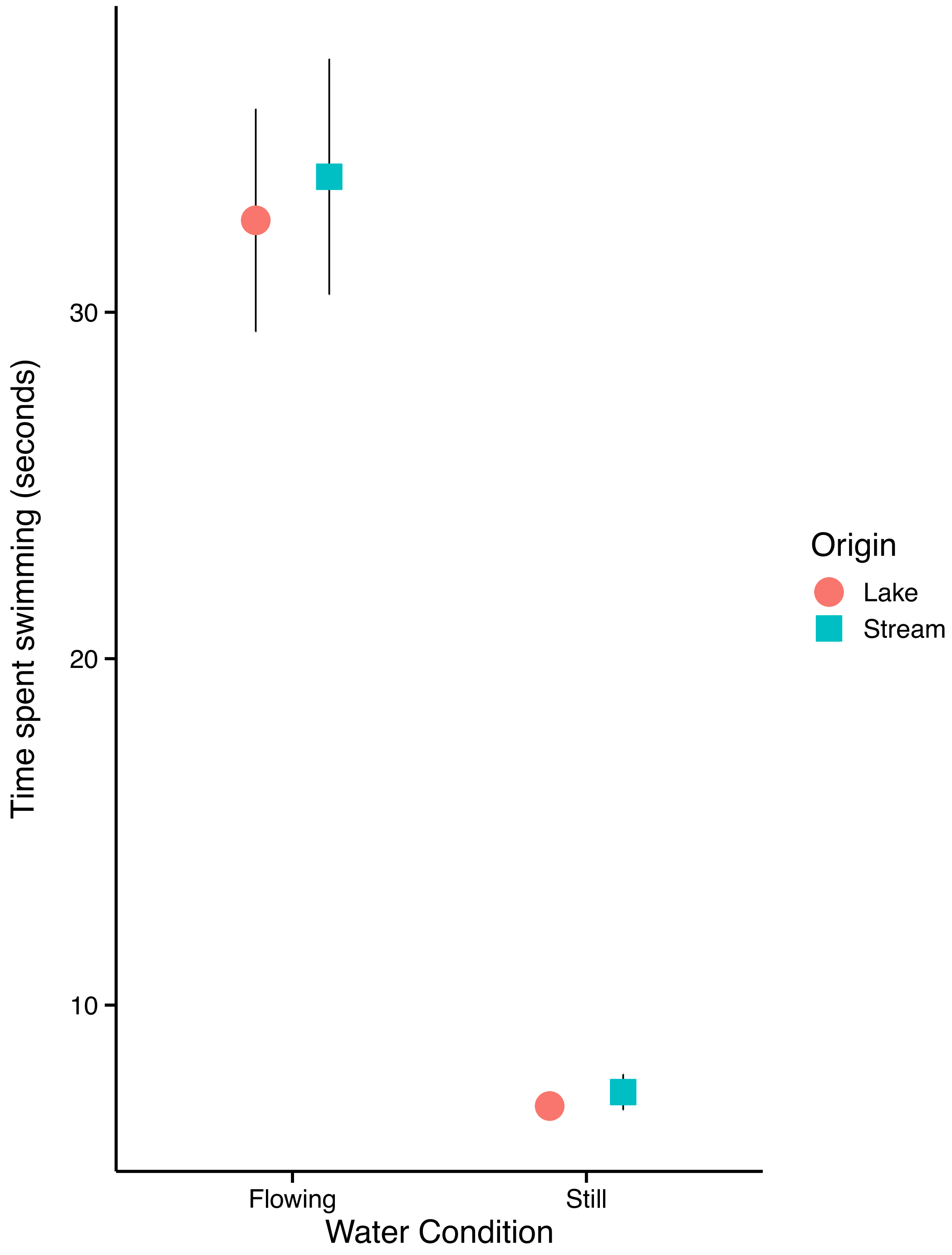

Figure 10. From the behavior assays, this plot shows the means and standard errors of each larvae type placed into each water condition. Overall, the larvae swam more in the flowing condition than the still water condition regardless of origin. There were significant differences for performance based on condition (F= 121.339, p=<2e-16).

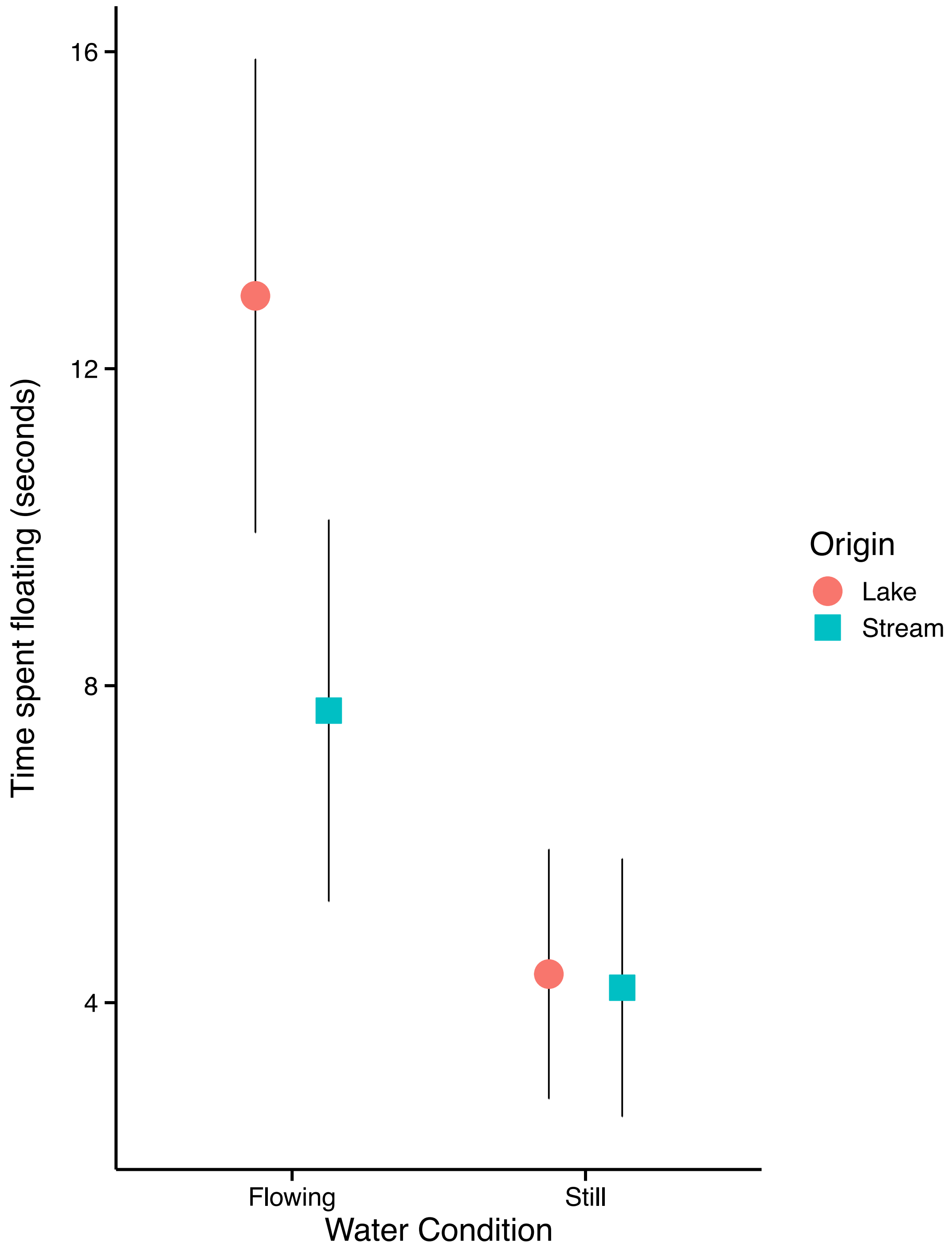

Figure 11. From the behavior assays, this plot shows the means and standard errors of each larvae type placed into each water condition. Overall, the larvae spent more time floating (not making any swim moves) in the flowing condition than the still water condition regardless of origin. There were significant differences for performance based on condition (F= 7.323, p=0.00748).

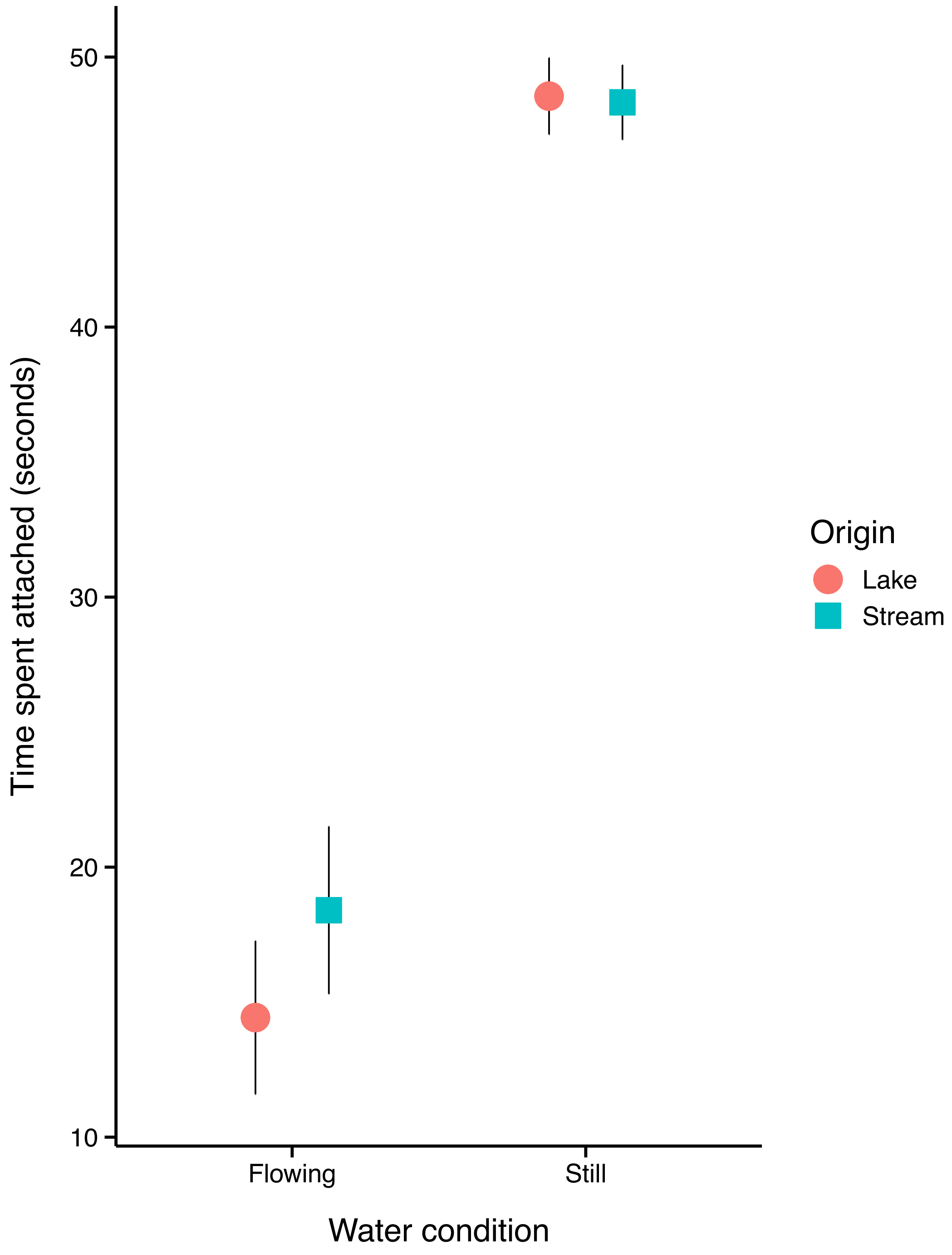

Figure 12. From the behavior assays, this plot shows the means and standard errors of each larvae type placed into each water condition. Overall, the larvae spent more time attached to a substrate in the flowing condition than the still water condition regardless of origin. There were significant differences for performance based on condition (F= 189.947, p=<2e-16).